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Can the addition of sugars boost non-starter lactic acid bacteria growth and accelerate cheese ripening?

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Introduction

The dynamics involved in the bacterial interaction during cheese ripening is not fully understood. The complexity as well as the variability related to cheese production are challenges which need to be investigated by researchers and industry. Significant cost saving could be achieved by accelerating the lysis of the starter lactic acid

bacteria (SLAB) and by increasing the growth of non starter lactic acid bacteria (NSLAB).

This study, using broth models and cheese trials, was performed to investigate the growth of NSLAB in presence of added N-acetylglucosamine, Ribose or N-acetylgalactosamine, with or without amino acids addition.

Materials and Methods

Before manufacturing the cheeses, broth models (adapted from Adamberg *et al.*, 2005) were performed to select the relevant sugars to be tested in the cheese trials (based on Ortakci *et al.*, 2015).

A) Selection of sugars: performed by measuring optical density (OD) in LM17 broth at 600 nm, every hour for 48h in a microtiter plate reader (Biotek Elx808, Vermont, USA) at controlled temperature (30° C). The broth models were used to investigate the growth potential of the selected commercial SLAB (a combination of *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*) starting at a level of 10⁵ CFU/mL. Sugars released in the cheese matrix during ripening are believed to be the energy source for NSLAB, therefore Ribose from starter DNA/RNA, N-acetylglucosamine and N-acetylmuramic acid from starter cell-wall as well as N-acetylgalactosamine from K-casein and milk fat globule membrane, were considered for the broth models. Only conditions where sugars were exclusively available for NSLAB were considered for the cheese trials.

B) Investigating effect of sugars in cheese matrices: Cheddar cheeses were manufactured, as show in Figure 1, using the same commercial SLAB. A level of 0.44 % of N-acetylglucosamine, Ribose or N-acetylgalactosamine was added to Cheddar cheeses, individually or in combination with amino acids (0.57 %) added at the milling step. Cheeses were vacuum packed and ripened at 10°C for a maximum period of six months.

C) Analytical design: Immediately after manufacture, and every month during a period of six months, Cheddar cheeses were analyzed using standard methods to measure in triplicate the aspects described in Figure 2.

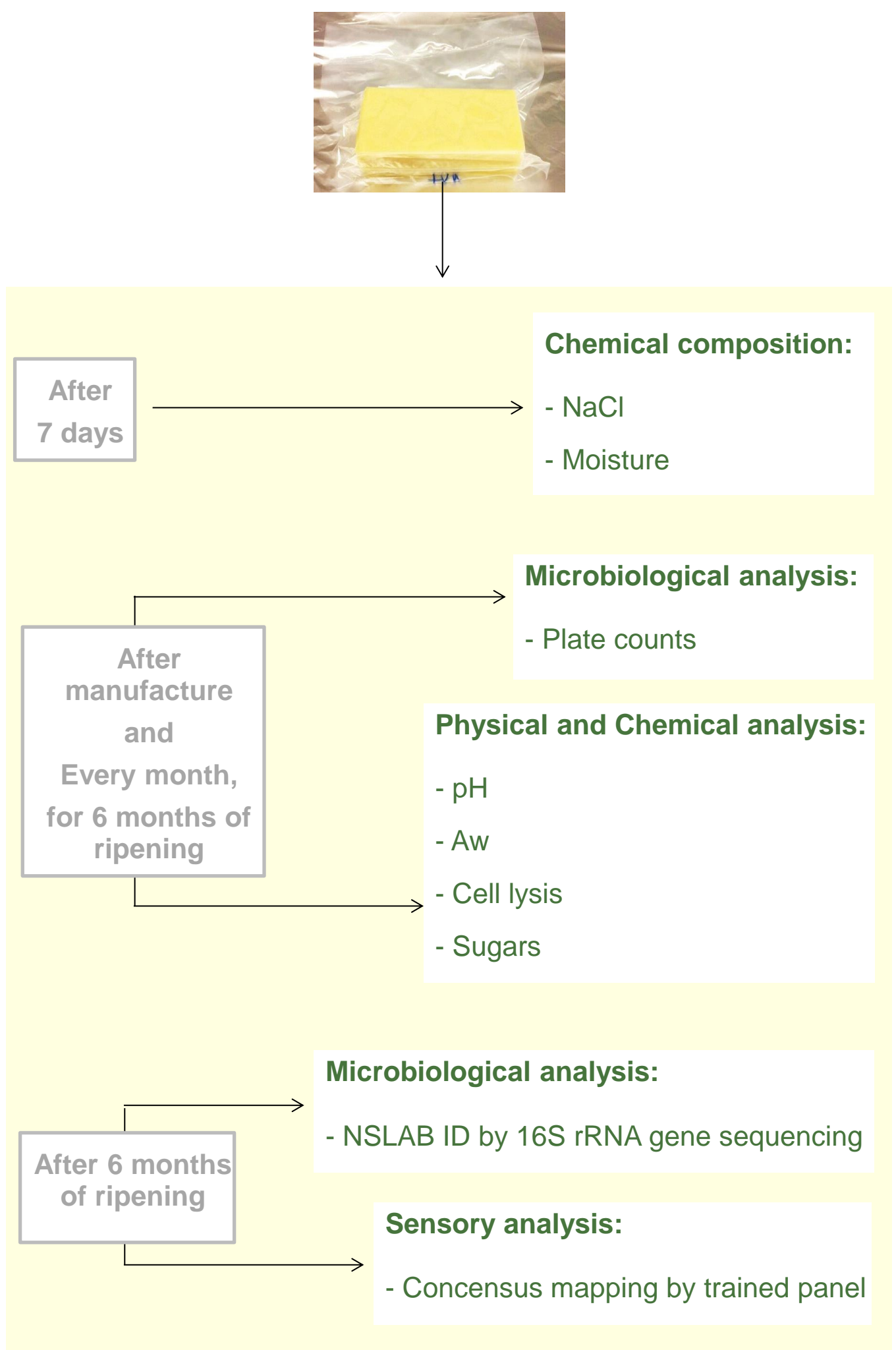


Figure 2: Overview of methods applied to investigate the effect of sugars on bacterial interaction during Cheddar cheese ripening

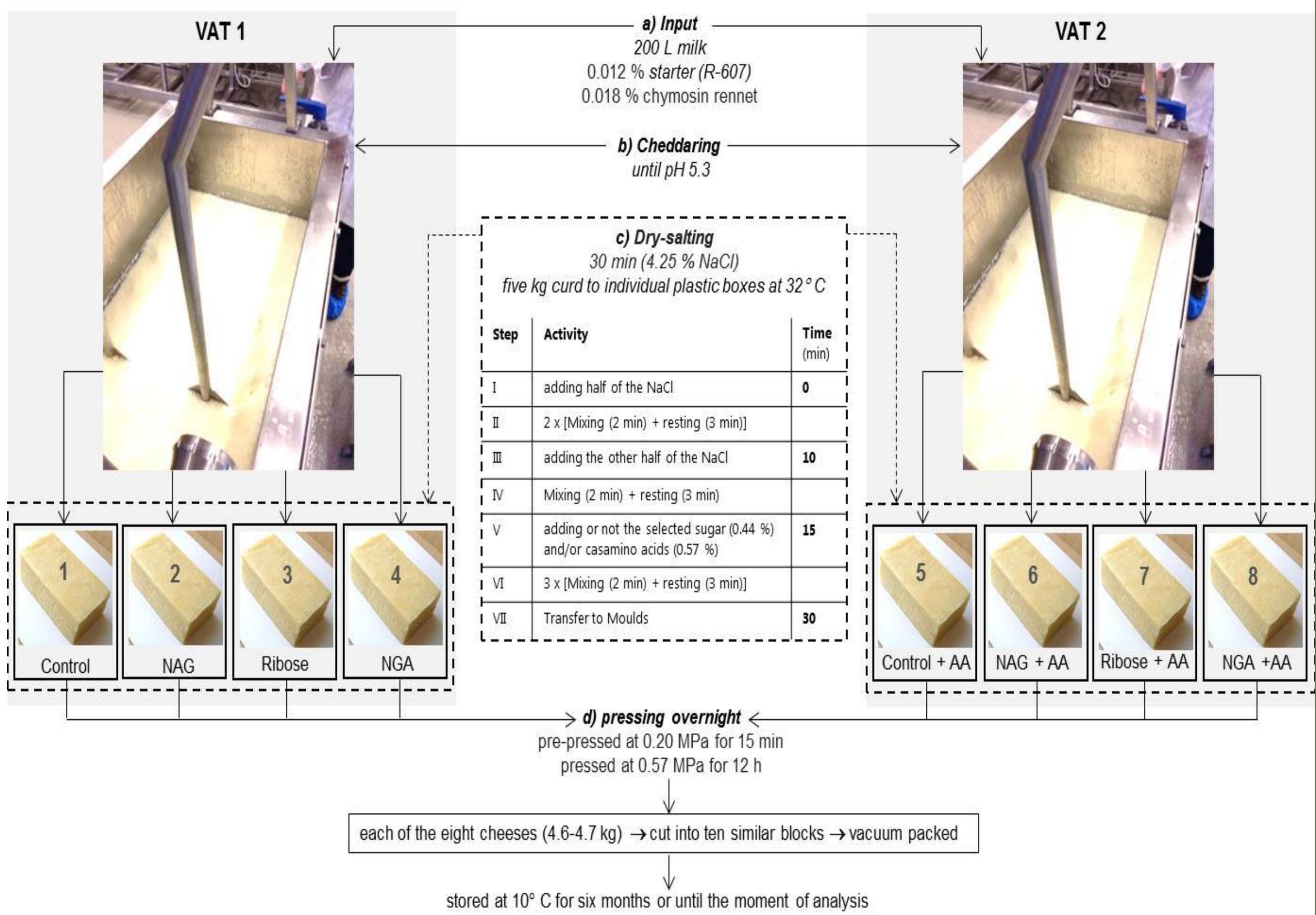
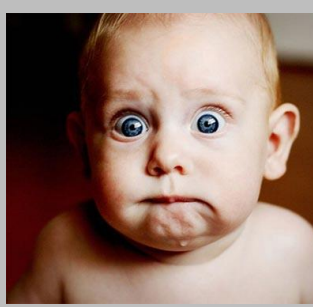


Figure 1: Schematic presentation of main steps to manufacture Cheddar cheeses without (Vat 1) or with Casamino acids - AA (Vat 2). The same batch of pasteurized bovine milk generated cheeses 1 and 5 (no addition of sugar), or with addition of 0.44 % sugar: cheeses 2 and 6 (N-acetylglucosamine), 3 and 7 (Ribose) and 4 and 8 (N-acetylgalactosamine)

Take-home messages when investigating effect of sugars on bacterial interaction during cheese ripening:



Bacteria act differently in a cheese matrix compared to broth models. Therefore, hypothesis need to be tested in both conditions for obtaining more reliable investigations



Adjunct cultures may be necessary when manufacturing cheeses, especially when using pasteurised milk and/or a new pilot plant, where NSLAB is not yet well established.



Sugars not preferred by starter cultures, such as N-acetyl galactosamine, seem to be good alternatives to boost NSLAB growth and accelerate cheese ripening.

Results

A) Selection of sugars through broth models: when comparing to the baseline, glucose support growth of the R-607 strains (Figure 3), both with and without 5 % NaCl added (salt content similar to high quality Cheddar cheese). Also N-acetylglucosamine (NAG) showed additional growth of the starter strains, but only when 5% NaCl was not added to the medium. All other tested sugars (in combination or not with 5 % NaCl) seemed not to increase the growth of SLAB, and could therefore, be considered as relevant candidates for the cheese trials.

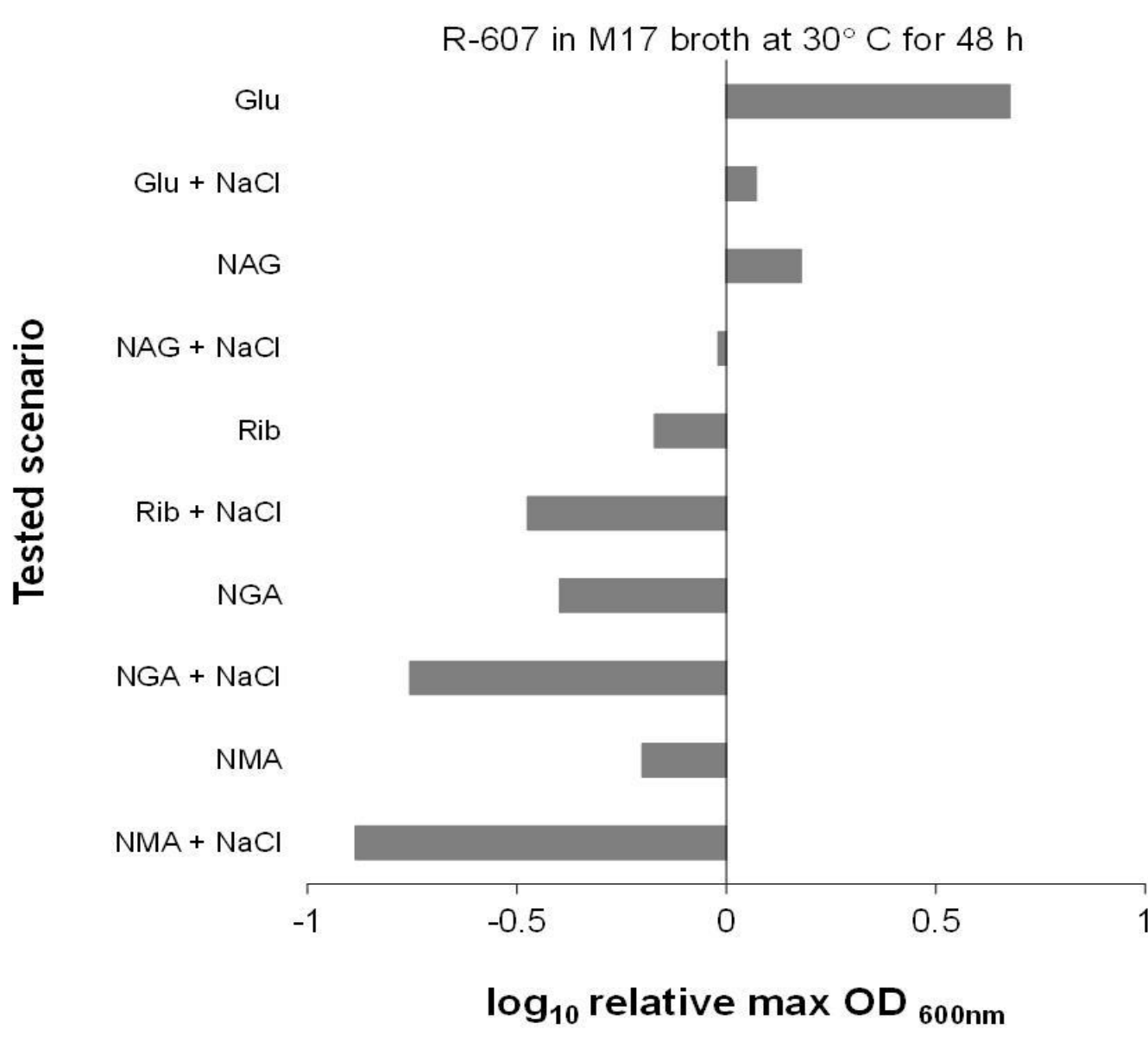


Figure 3: Log₁₀ relative maximum values (max) measured at OD_{600nm} of all scenarios testing effect of glucose (Glu), N-acetylglucosamine (NAG), ribose (Rib), N-acetylgalactosamine (NGA) or N-acetylmuramic acid (NMA) on growth of starter culture R-607 (inoculated at the level of 5.34 log₁₀ CFU g⁻¹) in M17 broth at 30° C for 48h. The scenarios refer to the sugars tested individually or in combination with NaCl (5 %) and are compared in relation to the maximum value of the baseline with no addition of sugar or NaCl (0.138 Log₁₀ max OD_{600nm})

B) Investigating effect of sugars in Cheddar cheese matrices:

Bacterial interaction: according to the bacterial development during the ripening of the cheeses (Figure 4), the added sugars had no relevant effect on the microbial communities' dynamics. Interestingly, results from 16S rRNA gene sequencing revealed no variation between the strains isolated from each of the cheese samples, or within the eight different cheeses.

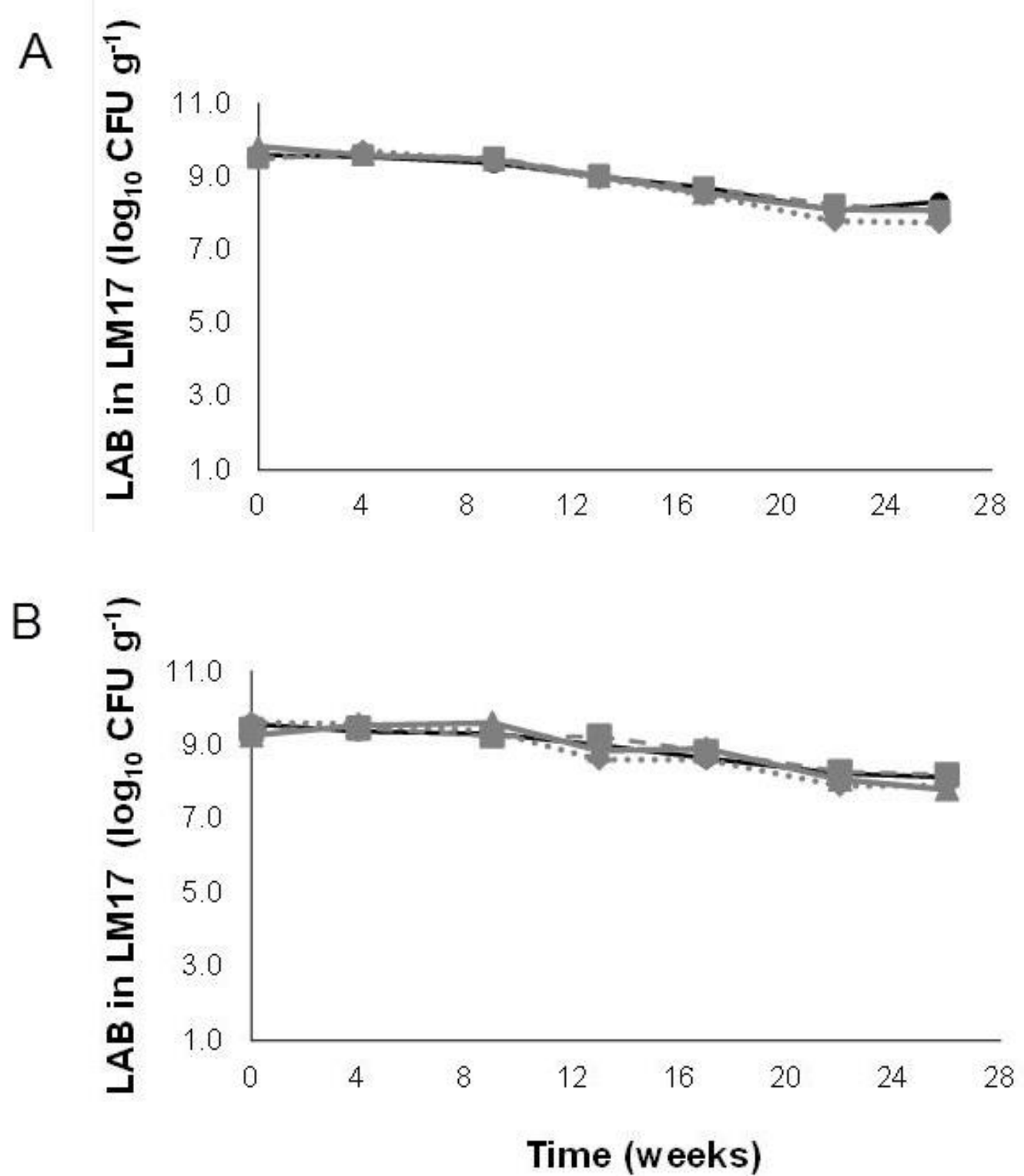


Figure 4: Changes in levels of lactic acid bacteria (LAB) during cheese ripening, cultivated aerobically on LM17 agar without (A) or with addition of amino acids (B). Vacuum packaged cheeses with no addition of sugars (in black, ○) were ripened at 10° C together with cheeses with addition of sugars (in grey) Ribose (◇), N-Acetylglucosamine (Δ) or N-Acetylgalactosamine (□)



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References

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Acknowledgements

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Quality parameters
(salt in moisture and pH)
inside expected range

Aw and cell lysis
(no difference between the cheeses)

Sensory analysis
(non harmonious)

Bacterial interaction
(no NSLAB found)

Sugar not used by SLAB
(N-acetyl galactosamine)

Take-home messages?